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MYELOID NEOPLASIA

Comment on Imgruet et al, page 790

The crux of Cux1 in myeloid neoplasms

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In this issue of *Blood*, Imgruet and colleagues investigate how loss of the tumor suppressor gene *Cux1* modulates DNA repair activity in the hematopoietic compartment and how this contributes to the pathogenesis of therapy-related myeloid neoplasms (tMNs).[1]

The blood system has tremendous regenerative capacity, which is called upon in response to blood loss, severe infections, and exposure to toxins, including systemic chemotherapy. Frequently, the blood system will reemerge from the ashes, but unlike the mythical phoenix, which rises reborn, the blood system comes back altered, and the fire will sometimes rage out of control. One way that this manifests is in higher rates of clonal hematopoiesis, myelodysplasia, and acute leukemia in the years following cancer treatment. This is a significant problem, because hematological malignancies that arise after therapy are difficult to treat and generally have poor outcomes.

CUX1 is a multifunctional protein, implicated in gene regulation, cell-cycle control, cell signaling, apoptosis, and the DNA damage response, and its role in cancer is predictably complex.[2] CUX1 is typically impaired in myeloid neoplasms; it is lost in around half of all cases of tMN, mostly through loss of chromosome 7, but sometimes through focal deletions or other mutations.[3] Mice engineered to have low *Cux1* expression are predisposed to myelodysplasia.[4] In line with earlier work in cell lines, the investigators show loss of Cux1 impairs the DNA damage response in primary murine hematopoietic stem and progenitor cells. Cux1-deficient progenitors expand after treatment with the alkylating agent *N*-ethyl-*N*-nitrosourea, and the stress encourages the rapid outgrowth of erythroleukemia, providing a new way to model this aggressive disease.

The investigators employ various functional assays to assess DNA repair activity in Cux1-deficient cells. They probe the response to DNA damaging agents, stain for DNA damage markers, and survey DNA strand breaks with COMET assays. None of these assays is perfect, but together they build a case that implicates Cux1 in modulating the DNA damage response. How does this occur? Again, it seems CUX1 acts at multiple levels. Imgruet et al suggest Cux1 recruits histone-modifying complexes to sites of damage and that this helps nucleate DNA repair. Some suggest a broader role, coordinating the expression of multiple DNA repair components, particularly in the ATM/ATR pathway.[5] Others suggest CUX1 directly modulates the activity of glycosylases, like OGG1, that repair oxidative damage.[6] More work is required to determine which of these activities is most crucial, or whether they work in concert.

The question then becomes, are CUX1-deficient cells accumulating more DNA damage? It is possible, but the answer is not yet definitive. By pulling together exome data from patients with various myeloid neoplasms, it appears *CUX1*-mutated samples have a slightly higher total mutation burden.[1,7] However, the difference is modest, and these comparisons are complicated by the low number of cases, the diverse disease spectrum, and differences in age, treatment history, and lifestyle factors. One way to answer this question would be to perform whole-genome sequencing on clonal cultures of blood cells to reveal the mutation burden associated with CUX1 deficiency, either in the mouse model or in material from patients.[8] Studying the resulting mutational signatures, during steady state and in response to stress, will help reveal any underlying DNA repair defect.

If there is more DNA damage, are these mutations driving disease progression and poor outcome? The prevailing view is that DNA damage provides more fuel for the fire. Here, the authors reveal the power of their mouse model, which allows them to transiently lower *Cux1* expression.[4] They show that reintroducing Cux1 rescues erythroid differentiation and prevents myeloid transformation, suggesting that any DNA damage that has accumulated is not enough to drive disease progression. This is exciting, because it suggests that drugs that restore CUX1 function, or that act downstream of this multifunctional regulator, may offer a way to treat the disease. Indeed, targeting altered signaling and survival pathways in CUX1-deficient cells seems to be a promising strategy.[9] Although encouraging, it will be important to pursue these questions in more relevant clinical models that mirror the complexity of the disease.

We are just beginning to appreciate the influence of cancer therapies on the blood system.[10] This understanding will grow as

we learn to model treatment response, and as we apply new technology, like cellular barcoding, single-cell transcriptomics, and mutational profiling. Together, these approaches will help to reveal how complex, multifunctional tumor suppressors, like CUX1, safeguard the blood system. Cytotoxic chemotherapy remains a mainstay of cancer therapy, indicating that we will be dealing with this problem for some time to come. By modeling treatment response, I am hopeful that we can learn to rekindle the hematopoietic system safely and avoid starting a raging inferno.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Wang et al, page 806

Live or die: PD-L1 delays neutrophil apoptosis

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In this issue of *Blood*, Wang et al uncover a key role that programmed death ligand 1 (PD-L1) plays in delaying neutrophil apoptosis at the site of inflammation by activation of the phosphatidylinositol 3-kinase (PI3K)-AKT survival pathway.[1]

Polymorphonuclear neutrophils (PMNs, or neutrophils) are the most abundant circulating leukocytes, constituting 60% to 70% of circulating white blood cells. PMNs are terminally differentiated and have a short lifespan, but are essential for innate immunity and host defense against microbes.[2] They are the first cells to be massively recruited at the site of infection where they recognize microbes via different receptors, inducing engulfment of the microbe into a phagosome.[3,4] Killing of microbes by PMNs occurs through the release, into the phagosome, of toxic agents such as reactive oxygen species and the content of granules (myeloperoxidase, glucosidases, proteases, and antibacterial peptides, etc). Microbes can also be trapped and killed by neutrophil extracellular traps (NETs).[5] Many processes such as apoptosis, NETosis, pyroptosis, and necroptosis can induce the death of neutrophils,[5] upon which they are phagocytized and eliminated by macrophages through a process called efferocytosis, resulting in the cleaning of the infection site. Thus, PMNs are critical anti-inflammatory components of the innate immune system as their physiological role is to resolve both infection and inflammation. Nevertheless, excessively activated or delayed apoptosis results in PMNs becoming harmful to surrounding tissues due to cell injury and continued inflammatory reaction, the driving factors for inflammatory disorders such as sepsis.[4]

Tissue neutrophils are believed to have longer lifespans than circulating PMNs, due to the presence of survival factors at the inflammatory site. Extended neutrophil lifespan through apoptosis suppression has been reported in patients with several inflammatory diseases and is associated with increased disease severity.[6] Neutrophils isolated from blood die through constitutive apoptosis, which can be either accelerated or inhibited by several agents. Inhibition of neutrophil apoptosis can contribute to inflammation; however, the factors leading to dysregulation of neutrophil apoptosis in inflammation are still not completely identified. In this issue, Wang et al demonstrate that PD-L1 plays a key role by delaying neutrophil apoptosis at the site of inflammation through the PI3K-AKT pathway (see figure).

Programmed cell death 1 (PD-1) protein, expressed on immune cells, is an immune checkpoint inhibitory receptor that triggers immunosuppressive signaling pathways.[7] PD-1 binds to PD-L1 or PD-L2 and blocks activating signals from T-cell receptors. PD-1/PD-L1 function as brakes to limit the adaptive immune response and the beneficial T-cell functions in cancer. PD-L1 is expressed on the plasma membrane of T and B cells and antigen-presenting cells.[7] Cancer cells also express PD-L1, which binds to the T-cell surface via PD-1 allowing them to escape host immune response. Thus, anti-PD-1/anti-PD-L1 antibodies have been used to treat various types of cancer. Previous studies have shown that PD-L1 expression on neutrophils increases in various inflammatory conditions.[8,9] In this new study, Wang et al show that PD-L1 overexpression in neutrophils from septic patients correlates with neutrophil survival. Silencing PD-L1 expression using small interfering RNA (siRNA) accelerated apoptosis